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HCV Genotypes in Morocco

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To determine the hepatitis C virus (HCV) genotypes circulating in Morocco, virus isolates from 105 chronically infected and 19 hemodialysis patients were examined using the line probe assay. Genotypes 1 and 2 only were found among Moroccan patients. Subtypes 1b (47.6%) and 2a/2c (37.1%) were the most common, whereas subtype 1a (2.8%) was less common. Among the hemodialysis patients, only genotype 1 was found with a prevalence of 68.4% for subtype 1b and 15.8% for the subtype 1a. It was also shown that the HCV genotypes distribution varies with age in both studied populations. Subtype 1b was most prevalent among older patients, whereas subtype 2a/2c was mainly found among younger ones. Although Morocco belongs to the African continent, the circulating HCV strains are similar to those observed in some American and European countries. ***J. Med. Virol. 52:396–398, 1997.***

KEY WORDS: chronic hepatitis; hemodialysis patients; PCR

INTRODUCTION

Hepatitis C virus (HCV) is the major cause of post-transfusion non-A, non-B hepatitis [Choo et al., 1989; Kuo et al., 1989]. This virus is associated with high risk of chronicity, cirrhosis, and hepatocellular carcinoma. Hepatitis C virus, a positive-sense single-stranded RNA [Choo et al., 1989], has a high spontaneous mutation rate (10^{-3} substitutions per site annually) [Ogata et al., 1991; Okamoto et al., 1992]. This high variability has led research groups to classify HCV into six major genotypes based on nucleotide sequence homology [Simmonds et al., 1993]. The distribution of HCV genotypes has been found to be dependent geographically [McOmish et al., 1994]. Generally, genotypes 1 and 2 are found in the United States, Japan, and Europe [Bréchot, 1995]; genotype 3 is mainly found in Thailand and Singapore [Bukh et al., 1993]; genotypes 4 and 5 have been met in the Middle East and in South Africa, respectively [Dusheiko et al., 1994; Ohro et al., 1994], and genotype 6 is found only in Hong Kong [Bukh et al., 1993; McOmish et al., 1994]. Recent studies suggest that the clinical features of liver disease depend on the HCV genotype. An association between certain genotypes and disease severity, liver cirrhosis, and primary liver cancer has been reported [Pozzato et al., 1991]. It is also noteworthy that the success of interferon treatment seems to be type or subtype related [Kohara et al., 1995]. These observations, therefore, make the identification of infecting HCV genotypes from different geographical zones of great interest. The principal objective of the present study was to identify the Moroccan circulating HCV isolates. Sera from hemodialysis patients and patients with chronic HCV infection were considered for genotyping and the relationship between age and genotype was determined.

MATERIALS AND METHODS

Patients

Sera were collected from 105 seropositive patients chronically infected with hepatitis C virus (age range, 19–76 years) from the Biology Center of Pasteur Institute. Sera were also collected from 49 hemodialysis seropositive patients (age range, 21–69 years) from the Hemodialysis Centers of Rabat, Casablanca, and Agadir. The collected sera were aliquoted within 2 hours of blood collection (EDTA) and kept at −20°C until use.

HCV Genotyping

Virus RNA was detected using an HCV RNA PCR assay (Amplicor HCV; Roche Diagnostic Systems, Neuilly-sur-Seine, France) according to the manufacturer's instructions. The rTth (Amplicor HCV; Roche Diagnostic Systems, Neuilly sur Seine, France) was used for reverse transcription and amplification of the 5′ noncoding region of HCV genome and carried out on a thermal cycler (GeneAmp PCR System 2400). The primer pair [Young et al., 1993] allowing an amplification of 244 pb sequence was:

KY80: 5′-GCAGAAAGCGTCTAGCCATGGCGT-3′

KY78: 5′-CTCGCAAGCACCCTATCAGGCAGT-3′.

The PCR products were analysed directly for genotyp-

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TABLE I.
HCV Genotypes Prevalence Among Hemodialysis and Chronically Infected Patients According to Age

	Chronic		Hemodialysis	
	Analysed population	Young/old	Analysed population	Young/old
Genotype 1				
1a	2.8%	−/2.7%	15.8%	16.6%/15.4%
1b	47.6%	10%/72%	68.4%	50%/76.9%
1a/1b	4.7%	10%/1.3%	15.8%	33.3%/7.7%
Undetermined	1.9%			
Total	56.3%			
Genotype 2				
2a/2c	37.1%	80%/25%		
Undetermined	5.7%			
Total	42.8%			

ing using the line probe assay (LIPA, Innogenetics, S.A., Gent, Belgium).

RESULTS

The genotype of HCV isolates was first determined among chronically infected patients. One hundred and five patients with an age range between 19 and 75 years were included. Table I shows the distribution of HCV subtypes among the chronically infected patients: 47.6% were infected by subtype 1b, 2.8% by 1a, 4.7% by mixed subtypes 1a/1b, and 37.1% by subtypes 2a and/or 2c, and 7.6% have not been subtyped. Furthermore, the relationship between HCV genotypes and age was investigated; 82 chronically infected patients were taken for this study and the results are shown in Table I. Among the 82 patients, 10 were under 40 and 72 were over 40. We have shown that the subtypes 2a and/or 2c are prevalent (80%) among younger patients, whereas the subtype 1b is the most prevalent (72%), followed by subtypes 2a and/or 2c (25%), among older ones.

The genotypes of HCV strains from 49 hemodialysis patients were also investigated. The HCV RNA was detected in 24 out of 49 (48.9%) hemodialysis seropositive patients. Only genotype 1 was found: 68.4% had subtype 1b, 15.8% subtype 1a, and 15.8% others showed a mixed infection with subtypes 1a/1b. The subtype 1b was prevalent among both younger (50%) and older (76.9%) patients.

DISCUSSION

This study indicates the HCV genotypes distribution in Morocco. As shown in Table I, all isolates were classified into 2 genotypes: genotype 1 and 2, with a prevalence of 57% and 43%, respectively. Mainly, subtypes 1b and 2a/2c were predominant, with a prevalence of 47.6% and 37.1%, respectively, whereas subtype 1a was rarely found (2.8%) in the studied population. These prevalent Moroccan genotypes (1 and 2) have also been found in Italy, France, Japan, and United States [Bréchot, 1995] but are different from those observed on the African continent, in Egypt, South Africa [Bukh et al., 1993], and Central Africa [Xu et al., 1994], where HCV genotypes 4 and 5 are prevalent. The latter have not been found among the Moroccan patients studied. This unusual predominance of HCV genotypes 1 and 2 in African countries has also been observed in Cameroon [Nkengasong et al., 1995]. These results suggest a probable common origin of the Moroccan HCV isolates with the European ones rather than with the African ones. Indeed, this study shows that only genotype 1 is observed among the hemodialysis patients (Table I). It is to be noted that these patients are obtained from centers in three different cities. This discrepancy has also been observed in Taiwan [Chou et al., 1993], since the HCV strains isolated from infected patients were all of genotype 2, in contrast to hemodialysis patients, who had genotypes 2 and 3. This divergence in prevalence between these two patient groups can be explained by the use of a contaminated apparatus, which may propagate a single genotype and thus modify the prevalence of HCV genotypes in the hemodialysis group.

In other respects, it was noted that the age of the patients was related to HCV subtype, since subtype 1b is the most prevalent (mean range 74%) among older patients, either chronically infected or in hemodialysis (Table I). Subtype 1b has often been observed in older patients [Bréchot, 1995].

In the present study, some samples have not been subtyped or have appeared to have mixed infection. Direct sequencing of these unclassified genotypes may lead to the recognition of additional unidentified subtypes 1 and 2.

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